Boar Semen Collection, Processing, Cryopreservation and Artificial Insemination Protocol

Boar semen is collected using the hand glove technique and the gel fraction is removed using sterile gauze. The semen is diluted 1:1 (v:v) with Androhep Plus or Androstar (37 °C; Minitube, Verona, WI), cooled to 23 °C over 1 hour on the laboratory bench and then to 15 °C over 1.5 hours. The samples are then shipped to the repository at 15 °C for cryopreservation.

Upon receipt, the samples are centrifuged for 10 minutes at 800 x g at 15 °C. The supernatant is removed and the sperm pellets are consolidated by boar. The sperm concentration and motility are determined using spectrophotometry and a Hamilton Thorne motility analyzer (Beverly, MA), respectively (at least 5 fields of analysis and 500 sperm). The samples are first diluted to 2.8 x 10^9 sperm/mL with 15 °C LEY cooling extender (CE; see recipe section) and then cooled to 5 °C over 2 to 2.5 hours. The second dilution is performed drop-wise using 5 °C freezing extender (FE; see recipe section) over 5 minutes so that the samples are diluted to a final concentration of 1.4 x 10^9 sperm/mL. The samples are loaded into 0.5 mL CBS straws and frozen using the Cryo Bio System Mini Digitcool UJ400 (IMV Corporation, Minneapolis, MN) with the following curve: 5 ºC to -8 ºC at -20 ºC per minute, -8 ºC to -120 ºC at -69 ºC per minute, -120 ºC to -140 ºC at -20 ºC per minute. The samples are then plunged in liquid nitrogen for storage.

Samples are thawed for 8 seconds in a 70 °C water bath and motility analysis is performed as described previously.

Recipes and boar semen freezing protocol: Almlid and Johnson, Journal of Animal Science, 1988, 66:2899-2905.

Cooling extender (CE)-to prepare 1 liter:

- Place 88 grams of lactose in a 500 mL volumetric flask
- Fill volumetric flask to appropriate volume with DD water and mix well
- Pour out contents into a 2 liter beaker and retain the volumetric flask
- Add 300 mL of DD water to the original 500 mL volumetric flask, mix, and combine the contents with the other lactose solution in the 2 liter beaker
- Add 200 mL of fresh egg yolk to the lactose solution and mix well
- Centrifuge solution for 1 hour at 1700 x g

Freezing extender (FE)

- 6% Glycerol (by volume)

- 1.5% Equex paste (by volume)
- 92.5% CE (by volume)

Artificial insemination:

Deep intrauterine insemination of swine is performed according to Roca et al. (2003). Ten straws of frozen semen (1×10^9 sperm) are thawed, as described previously, and pooled. The sample is loaded into a syringe and inseminated as described by Martinez et al. (2001). After deposition of the sample into the uterine horns, an additional 2 mL of BTS is used to flush the insemination catheter and remove the remaining sperm sample.

Standard cervical/intrauterine insemination can also be performed using 2 insemination doses of 2×10^9 motile sperm diluted to a final volume of 80 mL in Beltsville Thawing Solution (Pursel and Johnson, 1975) and inseminated according to McNamara and Knox (2013).

References:

Martinez, E.A., Vazquez, J.M., Roca, J., Lucas, X., Gil, M.A., Parrilla, I., Vazquez, J.L., Day, B.N. 2001. Successful non-surgical deep intrauterine insemination with small numbers of spermatozoa in sows. Reproduction 122: 289-296.

McNamara, K.A., Knox R. V. 2013. Effect of using frozen-thawed boar sperm differing in post-thaw motility in the first and second inseminations on pregnancy establishment, litter size, and fetal paternity in relation to time of ovulation. J. Anim. Sci. 91:5637–5645.

Roca, J., Carvajal, G., Lucas, X., Vazquez, J.M., Martinez, E.A. 2003. Fertility of weaned sows after deep intrauterine insemination with a reduced number of frozen-thawed spermatozoa. Theriogenology 60:77-87.

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